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DATE: Tuesday, July 12, 2005

**Hide?** Set Name QueryHit Count*DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ*☐ L2 (nifedipine oxidase or p450 adj3 3A4 or p450 adj3 pcn1) same crystal\$8 3*DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ*☐ L1 (nifedipine oxidase or p450 adj3 3A4 or p450 adj3 pcn1) same crystal\$8 5

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### Search Results - Record(s) 1 through 5 of 5 returned.

☐ 1. Document ID: US 6335170 B1

L1: Entry 1 of 5

File: USPT

Jan 1, 2002

US-PAT-NO: 6335170

DOCUMENT-IDENTIFIER: US 6335170 B1

TITLE: Gene expression in bladder tumors

DATE-ISSUED: January 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Orntoft; Torben F.	DK 8230 Aabyhoj			DK

US-CL-CURRENT: [435/6](#); [435/91.1](#), [435/91.2](#), [536/23.1](#), [536/24.3](#), [536/24.31](#), [536/24.33](#)

ABSTRACT:

Methods for analyzing tumor cells, particularly bladder tumor cells employ gene expression analysis of samples. Gene expression patterns are formed and compared to reference patterns. Alternatively gene expression patterns are manipulated to exclude genes which are expressed in contaminating cell populations. Another alternative employs subtraction of the expression of genes which are expressed in contaminating cell types. These methods provide improved accuracy as well as alternative basis for analysis from diagnostic and prognostic tools currently available.

21 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	RMIC	Draw De
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☐ 2. Document ID: GB 2395718 A

L1: Entry 2 of 5

File: EPAB

Jun 2, 2004

PUB-NO: GB002395718A

DOCUMENT-IDENTIFIER: GB 2395718 A

TITLE: Crystal structure of cytochrome P450 3A4 and methods of use thereof

PUBN-DATE: June 2, 2004

## INVENTOR-INFORMATION:

NAME	COUNTRY
TICKLE, IAN JAMES	GB
VONRHEIN, CLEMENS	GB
WILLIAMS, PAMELA ANN	GB
JHOTI, HARREN	GB
KIRTON, STEWART BRIAN	GB

INT-CL (IPC): C12 N 9/02; G01 N 33/53

EUR-CL (EPC): C07K014/80; C12N009/02

## ABSTRACT:

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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☐ 3. Document ID: WO 2004038015 A1

L1: Entry 3 of 5

File: EPAB

May 6, 2004

PUB-NO: WO2004038015A1

DOCUMENT-IDENTIFIER: WO 2004038015 A1

TITLE: CRYSTAL STRUCTURE OF CYTOCHROME P450 3A4 AND ITS USE

PUBN-DATE: May 6, 2004

## INVENTOR-INFORMATION:

NAME	COUNTRY
TICKLE, IAN JAMES	GB
VONRHEIN, CLEMENS	GB
WILLIAMS, PAMELA ANN	GB
JHOTI, HARREN	GB
KIRTON, STEWART BRIAN	GB

INT-CL (IPC): C12 N 15/02; G06 F 17/50; A61 K 35/00

EUR-CL (EPC): C07K014/80; C12N009/02

## ABSTRACT:

CHG DATE=20040518 STATUS=O>The invention provides the crystal structure of the cytochrome P450 3A4 protein molecule. The structure is set out in Table 5. The structure may be used in to model the interaction of compounds such as pharmaceuticals with this protein, and to determine the structure of related cytochrome P450 molecules.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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## 4. Document ID: US 20050032119 A1

L1: Entry 4 of 5

File: DWPI

Feb 10, 2005

DERWENT-ACC-NO: 2005-151670

DERWENT-WEEK: 200516

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TITLE: Crystal of P450 3A4, useful for identifying candidate modulator of P450 3A4, assessing ability of compound to interact with P450 3A4 protein, obtaining representation of three-dimensional structure of crystal of cytochrome P450 3A4

INVENTOR: JHOTI, H; KIRTON, S ; TICKLE, I J ; VINKOVIC, D M ; VONRHEIN, C ; WILLIAMS, P A

PRIORITY-DATA: 2001GB-0008214 (April 2, 2001), 2001GB-0008212 (April 2, 2001)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>US 20050032119 A1</u>	February 10, 2005		371	G01N033/53

INT-CL (IPC): G01 N 33/48; G01 N 33/50; G01 N 33/53; G06 F 19/00

ABSTRACTED-PUB-NO: US20050032119A

## BASIC-ABSTRACT:

NOVELTY - A crystal of P450 3A4 (I), has an orthorhombic space group 1222, a space group space group P21212, a resolution better than 3.1 Angstrom , and the structure defined by the coordinates (C1) fully defined in the specification plus or minus a root mean square deviation from the C alpha atoms of not more than 1.5 Angstrom .

## DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a computer-based method (M1) for the analysis of the interaction of a molecular structure with P450 structure, involves providing a structure comprising a three-dimensional representation of P450 3A4 or its portion, where the representation comprises all or a portion of (C1) plus or minus a root mean square deviation from C alpha atoms of not more than 1.5 Angstrom , providing a molecular structure to be fitted to P450 3A4 structure and its selected coordinates, and fitting the molecular structure to P450 3A4 structure;

(2) a compound (II) having the modified structure identified by (M1);

(3) obtaining (M2) a structure of a target P450 protein of unknown structure, involves providing a crystal of the target P450;

(4) a computer-based method (M3) for the analysis of molecular structures, involves providing the coordinates of at least two atoms of P450 3A4 structure of (C1) plus or minus a root mean square deviation from the C alpha atoms of less than 1.5 Angstrom ;

(5) a computer-based method (M4) of rational drug design involves providing the coordinates of at least two atoms of P450 3A4 structure of (C1) plus or minus a root mean square deviation from the C alpha atoms of less than 1.5 Angstrom ;

(6) a computer system (III), intended to generate structures and/or perform optimisation of compounds which interact with P450 or its homolog or analog, complexes of P450 with compounds, or complexes of P450 homolog or analog with

compounds;

(7) providing (M5) data for generating structures and/or performing optimisation of compounds which interact with P450 or its homolog or analog;

(8) a computer-readable storage medium (V) comprising a data storage material encoded with a first set of computer-readable data comprising a Fourier transform of at least a portion of the structural coordinates for the P450 protein defined by (C1) plus or minus a root mean square deviation from the C alpha atoms of not more than 1.5 Angstrom or its selected coordinates;

(9) co-crystal (VI) of P450 3A4 and a ligand;

(10) a chimeric protein (VII) having a binding cavity which provides a substrate specificity substantially identical to that of P450 3A4 protein, where the chimeric protein binding cavity is lined by several atoms which correspond to selected P450 3A4 atoms lining the P450 3A4 binding cavity, the relative positions of several atoms corresponding to the relative positions, as defined by (C1) of the selected P450 3A4 atoms;

(11) a compound (VIII) identified, produced or obtainable by (I); and

(12) a computer-based method for identifying a candidate modulator of P450 3A4, involves employing a three-dimensional structure of P450 3A4, or its selected co-ordinates, identifying the candidate modulator by designing or selecting a compound for interaction with the binding cavity.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Modulator of P450 3A4 (claimed).

USE - (I) is useful for identifying a candidate modulator of P450 3A4, which involves employing a three-dimensional structure of P450 3A4, its sub-domain, or its several atoms, to characterize at least one P450 3A4 binding cavity, and identifying the candidate modulator by designing or selecting a compound for interaction with the binding cavity. (I) is useful for determining the structure of a protein, which involves providing (C1) or its selected coordinates, and either positioning the coordinates in the crystal unit cell of the protein so as to provide a structure for the protein, or assigning nuclear magnetic resonance (NMR) spectra peaks of the protein by manipulating the coordinates. (I) is useful for modifying the structure of a compound in order to alter its metabolism by a P450, which involves fitting a starting compound to one or more coordinates of at least one amino acid residue of the ligand-binding region of P450, modifying the starting compound structure to increase or decrease its interaction with the ligand-binding region. The ligand-binding region includes at least 4 of the residues. (I) is useful for modifying the structure of a compound in order to alter its, or another compounds, metabolism by P450, which involves fitting a starting compound to one or more coordinates of at least one amino acid residue of the peripheral binding region of P450, modifying the starting compound structure to increase or decrease its interaction with the peripheral binding region, where the peripheral binding region is defined as the P450 residues numbered as 213, 214 or 219. (I) is useful for designing the structure of a compound which binds to the peripheral binding region, in order to alter another compounds metabolism by P450, which involves fitting a starting compound to one or more coordinates of at least one amino acid residue of the peripheral binding region of P450, modifying the starting compound structure to increase or decrease its interaction with the peripheral binding region, where the peripheral binding region is defined as P450 residues numbered as 213, 214 or 219. The method further involves fitting a second compound to the ligand binding site of P450. (I) is useful for obtaining a representation of the three-dimensional structure of a crystal of cytochrome P450 3A4, which involves

providing the data of (C1) or its selected coordinates, and constructing a three-dimensional structure representing the coordinates. (I) is useful for predicting three-dimensional structures of P450 homolog or analog of unknown structure, which involves aligning a representation of an amino acid sequence of a target P450 protein of unknown three-dimensional structure with the amino acid sequence of the P450 of (C1) to match homologous regions of the amino acid sequences, modeling the structure of the matched homologous regions of target P450 of unknown structure on the corresponding regions of P450 structure as defined by (C1), and determining a conformation for the target P450 of unknown structure which substantially preserves the structure of the matched homologous regions. (I) is useful for assessing the ability of a compound to interact with P450 3A4 protein which involves obtaining or synthesizing the compound, forming a crystallized complex of a P450 3A4 protein and the compound, the complex diffracting X-rays for the determination of atomic coordinates of the complex to a resolution of better than 2.8 Angstrom , and analyzing the complex by X-ray crystallography to determine the ability of the compound to interact with the P450 3A4 protein. The method involves identifying a molecular structure or modulator by (I) (all claimed). (VIII) is useful for treating cancer.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KMC	Draw. De
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5. Document ID: GB 2408509 A, WO 2004038015 A1, GB 2395718 A, AU 2003274378 A1, GB 2395718 B

L1: Entry 5 of 5

File: DWPI

Jun 1, 2005

DERWENT-ACC-NO: 2004-440452

DERWENT-WEEK: 200536

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TITLE: Obtaining a representation of the 3-D structure of cytochrome P450 3A4 crystals, by providing data of structure factors used to generate electron density map of crystal structure and constructing electron density map of obtained data

INVENTOR: JHOTI, H; KIRTON, S B ; TICKLE, I J ; VONRHEIN, C ; WILLIAMS, P A

PRIORITY-DATA: 2003US-479448P (June 19, 2003), 2002US-421063P (October 25, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>GB 2408509 A</u>	June 1, 2005		000	C12N009/02
<u>WO 2004038015 A1</u>	May 6, 2004	E	357	C12N015/02
<u>GB 2395718 A</u>	June 2, 2004		000	C12N009/02
<u>AU 2003274378 A1</u>	May 13, 2004		000	C12N015/02
<u>GB 2395718 B</u>	January 19, 2005		000	C12N009/02

INT-CL (IPC): A61 K 35/00; C12 N 9/02; C12 N 15/02; G01 N 33/53; G06 F 17/50

ABSTRACTED-PUB-NO: WO2004038015A

BASIC-ABSTRACT:

NOVELTY - Obtaining (M1) a representation of the 3-dimensional structure of a crystal of cytochrome P450 3A4, involves providing the data (F1) of structure factors and phases used to generate electron density map of 3A4 crystal structures

as given in specification, and constructing an electron density map of F1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a computer-based method (M2) for the analysis of the interaction of a molecular structure with a P450 structure;

(2) a compound (I) having the modified structure identified using (M2);

(3) obtaining (M3) an electron density map of a target P450 protein of unknown structure;

(4) determining (M4) whether a compound is bound to P450 3A4 protein;

(5) determining an electron density map of a target protein which is, or is homologous to, 3A4, involves providing a crystal of the target protein, obtaining an X-ray diffraction of the protein, and generating an electron density map of the target protein by reference to the structure factor phase data of F1;

(6) a computer-based method (M5) for the analysis of molecular structures;

(7) a computer-based method (M6) of rational drug design;

(8) identifying (M7) a candidate modulator of P450 3A4;

(9) determining (M8) the structure of protein;

(10) determining (M9) the structure of a compound bound to P450 protein;

(11) a computer system (II), intended to generate structures and/or perform optimization of compounds which interact with P450, its homologs or analogs, complexes of P450 with compounds, or complexes of P450 homologs or analogs with compounds, comprising computer-readable data (CRD);

(12) providing (M10) data for generating structures and/or performing optimization of compounds which interact with P450, its homologs or analogs complexes of P450 with compounds or complexes of P450 homologs or analogs with compounds, involves establishing communication with a remote device containing CRD, as defined for (II);

(13) a computer-readable storage medium (CR);

(14) crystal (III) of P450 3A4, having an orthorhombic space group I222, and unit cell dimensions 78 Angstrom , 100 Angstrom , 132 Angstrom , 90 deg. , 90 deg. , 90 deg. , with a unit cell variability of 5% in all dimensions, having a resolution better than 3.1 Angstrom ;

(15) a crystal of P450 protein having the structure defined by the coordinates of C1 plus or minus a root mean square deviation from the C alpha atoms of not more than 1.5 Angstrom ;

(16) predicting 3-dimensional structures of P450 homologs or analogs of unknown structure;

(17) a chimeric protein having a binding cavity which provides a substrate specificity substantially identical to that of P450 3A4 protein, where the chimeric protein binding cavity is lined by several atoms which correspond to selected P450 3A4 atoms lining the P450 3A4 binding cavity, the relative positions of the several atoms corresponding to the relative positions, as defined by C1 of selected P450 3A4 atoms;

(18) assessing the ability of a compound to interact with P450 3A4 protein;

(19) a compound (C2) identified, produced, or obtainable by (M6) or (M7); and

(20) a computer-based method for identifying a candidate modulator of P450 3A4, involves employing a 3-dimensional structure of P450 3A4, or its selected coordinates, where the 3-dimensional structure is defined by atomic coordinate data as defined C1, and identifying the candidate modulator by designing or selecting a compound for interaction with the binding cavity.

ACTIVITY - Cytostatic.

No biological data given.

MECHANISM OF ACTION - Modulator of P450 3A4 (claimed).

USE - (M1) is useful for obtaining representation of the 3-dimensional structure of a crystal of cytochrome P450 3A4, where the crystal structure is useful in modeling the interaction of a compound with the protein, and in drug design. (M2) or (M5)-(M9) is useful for preparing a composition, which involves identifying a molecular structure or modulator by the above-mentioned methods, and admixing the molecule with a carrier. (M2) or (M5)-(M9) is useful for producing a medicament, pharmaceutical composition or drug, which involves identifying molecular structure or modulator by the above-mentioned methods, and preparing a medicament, pharmaceutical composition or drug containing the optimized modulator molecule, where the compound or composition is useful in medicine (claimed).

A pharmaceutical composition comprising C2 and a carrier is useful for treating or preventing cancer.

ADVANTAGE - The 3A4 structure of (M1) is suitable for soaking in ligands and hence enables determination of complex structures. The residues in binding pocket are well resolved.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	RMK	Draw Da
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Clear	Generate Collection	Print	Fwd Refs	Bkwd Refs	Generate OACS
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Terms	Documents
(nifedipine oxidase or p450 adj3 3A4 or p450 adj3 pcn1) same crystal\$8	5

Display Format:

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## STN SEARCH

10/690,991

FILE 'HOME' ENTERED AT 12:20:13 ON 12 JUL 2005

=&gt; file .nash

=&gt; s (p450 (3w) 3A4 or P450 (3w) pcn1 or nifedipine oxidase) and crystal?

L1 7 FILE MEDLINE  
L2 14 FILE CAPLUS  
L3 22 FILE SCISEARCH  
L4 1 FILE LIFESCI  
L5 18 FILE BIOSIS  
L6 24 FILE EMBASE

TOTAL FOR ALL FILES

L7 86 (P450 (3W) 3A4 OR P450 (3W) PCN1 OR NIFEDIPINE OXIDASE) AND  
CRYSTAL?

=&gt; dup rem l7

PROCESSING COMPLETED FOR L7

L8 56 DUP REM L7 (30 DUPLICATES REMOVED)

=&gt; s l7 not 2003-2005/py

TOTAL FOR ALL FILES

L15 48 L7 NOT 2003-2005/PY

=&gt; dup rem l15

PROCESSING COMPLETED FOR L15

L16 32 DUP REM L15 (16 DUPLICATES REMOVED)

=&gt; d ibib abs 1-32

L16 ANSWER 1 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2003006470 EMBASE Full-text

TITLE: A ligand-based approach to understanding selectivity of  
nuclear hormone receptors PXR, CAR, FXR, LXR $\alpha$ , and  
LXR $\beta$ .

AUTHOR: Ekins S.; Mirny L.; Schuetz E.G.

CORPORATE SOURCE: S. Ekins, Concurrent Pharmaceuticals Inc., Fort Washington,  
PA 19034, United States. [sekins@concurrentpharma.com](mailto:sekins@concurrentpharma.com)  
SOURCE: Pharmaceutical Research, (1 Dec 2002) Vol. 19, No. 12, pp.  
1788-1800.

Refs: 118

ISSN: 0724-8741 CODEN: PHREEB

COUNTRY: United States

DOCUMENT TYPE: Journal; General Review

FILE SEGMENT: 002 Physiology

037 Drug Literature Index

038 Adverse Reactions Titles

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 20030116

Last Updated on STN: 20030116

AB In recent years discussion of nuclear hormone receptors, transporters, and drug-metabolizing enzymes has begun to take place as our knowledge of the overlapping ligand specificity of each of these proteins has deepened. This ligand specificity is potentially valuable information for influencing future drug design, as it is important to avoid certain enzymes or transporters in order to circumvent potential drug-drug interactions. Similarly, it is critical that the induction of these same proteins via nuclear hormone receptors is avoided, as this can result in further toxicities. Using a ligand-based approach in this review we describe new and previously published computational models for PXR, CAR, FXR, LXR $\alpha$ , and LXR $\beta$  that may help in understanding the complexity of interactions between transporters and enzymes. The value of these types of models is that they may enable us to design molecules to selectively modulate pathways for therapeutic effect and in addition predict the potential for drug interactions more reliably. Simultaneously, we might learn which came first: the transporter, the enzyme, or the nuclear hormone receptor?

L16 ANSWER 2 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on

STN

ACCESSION NUMBER: 2002:522186 SCISEARCH Full-text  
THE GENUINE ARTICLE: 565AP  
TITLE: 7-benzoyloxyquinoline oxidation by P450eryF A245T: Finding of a new fluorescent substrate probe  
AUTHOR: Khan K K (Reprint); Halpert J R  
CORPORATE SOURCE: Univ Texas, Med Branch, Dept Pharmacol & Toxicol, 301 Univ Blvd, Galveston, TX 77555 USA (Reprint); Univ Texas, Med Branch, Dept Pharmacol & Toxicol, Galveston, TX 77555 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (JUN 2002) Vol. 15, No. 6, pp. 806-814.  
ISSN: 0893-228X.  
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 30  
ENTRY DATE: Entered STN: 12 Jul 2002  
Last Updated on STN: 12 Jul 2002

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The main objective of the present study was to find a fluorescent substrate probe for cytochrome P450eryF (P450eryF). P450eryF is a bacterial P450 that catalyzes the hydroxylation of 6-deoxyerythronolide B at the 6S position, a necessary step in the biosynthesis of erythromycin. The lack of a conserved threonine residue in the I-helix, in contrast to other P450s, makes P450eryF unable to oxidize other substrates. A recent study [Xiang et al. (2000) J. Biol. Chemical 275, 35999-36006] has shown that the substitution of Ala-245 by threonine confers on P450eryF significant testosterone hydroxylase activity. Therefore, we investigated various known fluorescent P450 substrates with P450eryF wild-type as well as two mutants, A245S and A245T. Among the various fluorescent compounds tested, 7-benzoyloxyquinoline (7-BQ) was found to be the most suitable probe for P450eryF A245T, with rates of oxidation being lower for A245S and wild-type enzyme. The steady-state kinetics of 7-BQ oxidation by A245T are sigmoidal ( $V_{max} = 0.71$  nmol/min/nmol,  $n = 2.18$ , and  $S_{50} = 132$   $\mu$ M).  $\alpha$ -Naphthoflavone ( $\alpha$ -NF), a well-known activator of CYP3A4, did not stimulate 7-BQ oxidation by A245T, although the  $S_{50}$  value for  $\alpha$ -NF binding to wild-type P450eryF was similar to P450 3A4. Interestingly, spectral binding studies of wild-type P450eryF and A245T with ketoconazole and miconazole showed differential binding behaviors. Titration of wild-type with ketoconazole and miconazole and of A245T with miconazole showed the expected type-II binding. However, titration of A245T with ketoconazole produced a spectrum similar to type-I. Inhibition studies showed that both ketoconazole and miconazole are able to inhibit 7-BQ oxidation by A245T, although miconazole showed a slightly higher potency. In brief, the present study reports the discovery of 7-BQ as the first fluorescent and only the second unnatural substrate, and of miconazole as an effective P450eryF inhibitor.

L16 ANSWER 3 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:207398 SCISEARCH Full-text  
THE GENUINE ARTICLE: 525FL  
TITLE: Midazolam oxidation by cytochrome P450 3A4 and active-site mutants: an evaluation of multiple binding sites and of the metabolic pathway that leads to enzyme inactivation  
AUTHOR: Khan K K (Reprint); He Y Q; Domanski T L; Halpert J R  
CORPORATE SOURCE: Univ Texas, Med Branch, Dept Pharmacol & Toxicol, Route 1031, 301 Univ Blvd, Galveston, TX 77555 USA (Reprint); Univ Texas, Med Branch, Dept Pharmacol & Toxicol, Galveston, TX 77555 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: MOLECULAR PHARMACOLOGY, (MAR 2002) Vol. 61, No. 3, pp. 495-506.  
ISSN: 0026-895X.  
PUBLISHER: AMER SOC PHARMACOLOGY EXPERIMENTAL THERAPEUTICS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 40  
ENTRY DATE: Entered STN: 15 Mar 2002  
Last Updated on STN: 15 Mar 2002

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Midazolam (MDZ) oxidation by recombinant CYP3A4 purified from Escherichia coli and 30 mutants generated at 15 different substrate recognition site positions has been studied to determine the role of individual residues in regioselectivity and to investigate the possible existence of multiple binding sites. Initial results showed that oxidation of MDZ by CYP3A4 causes time- and concentration-dependent enzyme inactivation with  $K_m$  and  $k_{inact}$  values of 5.8  $\mu$ M and 0.15  $\text{min}^{-1}$ , respectively. The different time courses of MDZ hydroxylation by mutants that predominantly formed 1'-OH MDZ as opposed to 4-OH MDZ provided strong evidence that the 1'-OH MDZ pathway leads to CYP3A4 inactivation. Correlational analysis of 1'-OH formation versus 4-OH formation by the mutants supports the inference that the two metabolites result from the binding of MDZ at two separate sites. Thus, substitution of residues Phe-108, Ile-120, Ile-301, Phe-304, and Thr-309 with a larger amino acid caused an increase in the ratio of 1'-OH/4-OH MDZ formation, whereas substitution of residues Ser-119, Ile-120, Leu-210, Phe-304, Ala-305, Tyr-307, and Thr-309 with a smaller amino acid decreased this ratio. Kinetic analyses of nine key mutants revealed that the alteration in regioselectivity is caused by a change in kinetic parameters ( $V_{max}$  and  $K_M$ ) for the formation of both metabolites in most cases. The study revealed the role of various active-site residues in the regioselectivity of MDZ oxidation, identified the metabolic pathway that leads to enzyme inactivation, and provided an indication that the two proposed MDZ binding sites in CYP3A4 may be partially overlapping.

L16 ANSWER 4 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:83704 BIOSIS Full-text

DOCUMENT NUMBER: PREV200300083704

TITLE: Molecular modelling of the human glucocorticoid receptor (hGR) ligand-binding domain (LBD) by homology with the human estrogen receptor alpha (hERalpha) LBD: Quantitative structure-activity relationships within a series of CYP3A4 inducers where induction is mediated via hGR involvement.

AUTHOR(S): Lewis, D. F. V. [Reprint Author]; Ogg, M. S.; Goldfarb, P. S.; Gibson, G. G.

CORPORATE SOURCE: School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK  
d.lewis@surrey.co.uk

SOURCE: Journal of Steroid Biochemistry and Molecular Biology, (October 2002) Vol. 82, No. 2-3, pp. 195-199. print.  
CODEN: JSBBEZ. ISSN: 0960-0760.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 6 Feb 2003

Last Updated on STN: 6 Feb 2003

AB The results of homology modelling of the human glucocorticoid receptor (hGR) ligand-binding domain (LBD) based on the ligand-bound domain of the human estrogen receptor alpha (hERalpha) are reported. It is shown that known hGR ligands which induce the human cytochrome P450 enzyme CYP3A4 are able to fit the putative ligand-binding site of the nuclear hormone receptor and form hydrogen bonds with key amino acid residues within the binding pocket. Quantitative structure-activity relationships (QSARs) have been derived for hGR-mediated CYP3A4 induction which involve certain molecular structural and physicochemical properties of the ligand themselves, yielding good correlations ( $R=0.96-0.98$ ) with fold induction of CYP3A4 known to be mediated via hGR involvement.

L16 ANSWER 5 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2002:85222 CAPLUS Full-text

DOCUMENT NUMBER: 136:334712

TITLE: Formation of a novel quinone epoxide metabolite of troglitazone with cytotoxic to HepG2 cells

AUTHOR(S): Yamamoto, Yui; Yamazaki, Hiroshi; Ikeda, Tomoko; Watanabe, Terumi; Iwabuchi, Haruo; Nakajima, Miki; Yokoi, Tsuyoshi

CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, 920-0934, Japan

SOURCE: Drug Metabolism and Disposition (2002), 30(2), 155-160  
CODEN: DMDSAI; ISSN: 0090-9556

PUBLISHER: American Society for Pharmacology and Experimental Therapeutics

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Troglitazone, an oral antidiabetic drug, was reported to cause adverse hepatic effects in certain individuals, leading to its withdrawal from the market. After incubation of troglitazone (100 µM) with the human hepatoma cell line, HepG2 cells, and human primary hepatocytes for 48 to 72 h, an unknown peak was detected in the cell culture. The formation of this peak from troglitazone (100 µM) was also catalyzed by expressed CYP3A4, and further HPLC anal. revealed that there were three metabolites (metabolite A, B, and C) in the peak. The major metabolite, metabolite C (M-C) was identified as an epoxide of a quinone metabolite of troglitazone by comparison with a synthetic authentic standard using tandem mass spectrometry, 1H NMR, and 13C NMR analyses. The other two metabolites (M-A and M-B) were stereoisomers with the same mol. weight as M-C, probably produced from M-C by intramol. rearrangement at the epoxide moiety. M-C showed a weak cytotoxicity in HepG2 cells at low concns., as assessed by the crystal violet-staining assay. Since epoxides are generally regarded as the chemical reactive species, M-C may play a role in idiosyncrasy of troglitazone hepatotoxicity via individual differences either in the formation or degradation of this metabolite.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 6 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2002156875 EMBASE Full-text  
TITLE: Structural insights into the promiscuity and function of the human pregnane X receptor.  
AUTHOR: Watkins R.E.; Noble S.M.; Redinbo M.R.  
CORPORATE SOURCE: M.R. Redinbo, Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599-3290, United States. redinbo@unc.edu  
SOURCE: Current Opinion in Drug Discovery and Development, (2002) Vol. 5, No. 1, pp. 150-158.  
Refs: 49  
ISSN: 1367-6733 CODEN: CODDFP  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 030 Pharmacology  
037 Drug Literature Index  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20020516  
Last Updated on STN: 20020516

AB The pregnane X receptor (PXR) is a promiscuous nuclear receptor that responds to a wide variety of drugs, xenobiotics and endogenous compounds, and plays a critical role in mediating drug-drug interactions in humans. PXR is the master regulator of the expression of the CYP3A4 gene, which encodes for the most abundant and promiscuous drug-metabolizing enzyme in humans. PXR also regulates the expression of other genes involved in xenobiotic metabolism, including CYP2C8, CYP2C9, CYP2B6, GSTA2 and MDR1, as well as genes critical to bile acid metabolism. While PXR functions as a xenobiotic sensor in numerous vertebrates, its relatively low sequence conservation across species causes the PXR from different organisms to respond to distinct subsets of xenobiotics. Thus, PXR promiscuity is directed and not random. The recent determination of crystal structures of the ligand binding domain of human PXR has provided the first detailed molecular view of this promiscuous receptor, and has advanced our understanding of its varied biological functions. We review the evidence establishing the binding promiscuity of PXR and its directed specificity in different species, and analyze the structural determinants of these characteristics. In addition, we examine the relationship between the interaction of PXR with ligands and the manner in which CYP3A4 is thought to bind to substrate molecules. The accumulating structural and functional data on PXR may facilitate the development of improved methods for in vitro, in vivo and in silico screening for PXR activation.

L16 ANSWER 7 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2002141882 EMBASE Full-text  
TITLE: Molecular modeling of human cytochrome P450-substrate interactions.  
AUTHOR: Lewis D.F.V.  
CORPORATE SOURCE: D.F.V. Lewis, Sch. of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey GU2 7XH, United Kingdom. d.lewis@surrey.ac.uk  
SOURCE: Drug Metabolism Reviews, (2002) Vol. 34, No. 1-2, pp. 55-67.

Refs: 22  
 ISSN: 0360-2532 CODEN: DMTRAR  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 030 Pharmacology  
 037 Drug Literature Index  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English  
 ENTRY DATE: Entered STN: 20020502  
 Last Updated on STN: 20020502

AB The results of homology modeling of 10 human cytochrome P450 (CYP) enzymes involved in the Phase 1 metabolism of drugs and other foreign compounds are reported. The models have been constructed from the CYP102 hemoprotein domain template for which the substrate-bound crystallographic coordinates are available. Selective substrates of individual human P450s: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP4A11 are all shown to fit within the corresponding enzymes' active sites in such a manner that is consistent with reported experimental data for both known pathways of substrate metabolism and from the results of site-directed mutagenesis, either in those particular human P450 enzymes concerned or for ones within the same subfamily. The self-consistency of these homology models indicates that they may have potential utility for the pre-screening of novel drug structures.

L16 ANSWER 8 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:442601 CAPLUS Full-text  
 DOCUMENT NUMBER: 137:290104  
 TITLE: Quantitative structure-activity relationships for inducers of cytochromes P450 and nuclear receptor ligands involved in P450 regulation within the CYP1, CYP2, CYP3 and CYP4 families  
 AUTHOR(S): Lewis, D. F. V.; Jacobs, M. N.; Dickins, M.; Lake, B. G.  
 CORPORATE SOURCE: School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK  
 SOURCE: Toxicology (2002), 176(1-2), 51-57  
 CODEN: TXCYAC; ISSN: 0300-483X  
 PUBLISHER: Elsevier Science Ltd.  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB The results of quant. structure-activity relationships (QSARs) are reported for several series of cytochrome P 450 inducers, including those which also act as ligands for the various nuclear receptors involved in regulation of the relevant P 450 genes, namely, CYP1, CYP2, CYP3 and CYP4. In several examples presented, the QSARs are consistent with homol. modeling studies of the nuclear receptor ligand-binding domains (LBDs) based on available crystal structures of the estrogen and peroxisome proliferator-activated receptors' LBDs. Good correlations (R=0.91-0.99) are found between various structural parameters and biol. activity (either in the form of P 450 induction or ligand-binding affinity) for the Ah receptor (AhR), human estrogen receptor  $\alpha$  (hER $\alpha$ ), human glucocorticoid receptor (hGR) and the rat peroxisome proliferator-activated receptor  $\alpha$  (rPPAR $\alpha$ ).

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 9 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2002:106511 CAPLUS Full-text  
 DOCUMENT NUMBER: 137:15090  
 TITLE: The PXR crystal structure: the end of the beginning  
 AUTHOR(S): Ekins, Sean; Schuetz, Erin  
 CORPORATE SOURCE: Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN, 46285, USA  
 SOURCE: Trends in Pharmacological Sciences (2002), 23(2), 49-50  
 CODEN: TPHSDY; ISSN: 0165-6147  
 PUBLISHER: Elsevier Science Ltd.  
 DOCUMENT TYPE: Journal; General Review  
 LANGUAGE: English

AB A review. The pregnane X receptor (PXR) has a key role in the regulation of both drug metabolism and drug efflux by activating the expression of genes encoding cytochrome P 450 enzymes and drug efflux transporters, resp. Thus, coadministration of a PXR ligand and drugs handled by such enzymes or transporters leads to drug interactions. The need to

identify drugs in development with the least potential to cause adverse drug interactions has led to the recent solving of the crystal structure of the human PXR ligand binding domain. The recent solving of the crystal structure of the pregnane X receptor provides a better model to predict drug-drug interactions at an earlier stage of drug development.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 10 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:338762 CAPLUS Full-text

DOCUMENT NUMBER: 134:362292

TITLE: Methods of determining individual hypersensitivity to a pharmaceutical agent from gene expression profile

INVENTOR(S): Farr, Spencer

PATENT ASSIGNEE(S): Phase-1 Molecular Toxicology, USA

SOURCE: PCT Int. Appl., 222 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001032928	A2	20010510	WO 2000-US30474	20001103
WO 2001032928	A3	20020725		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 1999-165398P P 19991105  
US 2000-196571P P 20000411

AB The invention discloses methods, gene databases, gene arrays, protein arrays, and devices that may be used to determine the hypersensitivity of individuals to a given agent, such as drug or other chemical, in order to prevent toxic side effects. In one embodiment, methods of identifying hypersensitivity in a subject by obtaining a gene expression profile of multiple genes associated with hypersensitivity of the subject suspected to be hypersensitive, and identifying in the gene expression profile of the subject a pattern of gene expression of the genes associated with hypersensitivity are disclosed. The gene expression profile of the subject may be compared with the gene expression profile of a normal individual and a hypersensitive individual. The gene expression profile of the subject that is obtained may comprise a profile of levels of mRNA or cDNA. The gene expression profile may be obtained by using an array of nucleic acid probes for the plurality of genes associated with hypersensitivity. The expression of the genes predetd. to be associated with hypersensitivity is directly related to prevention or repair of toxic damage at the tissue, organ or system level. Gene databases arrays and apparatus useful for identifying hypersensitivity in a subject are also disclosed.

L16 ANSWER 11 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:9408 CAPLUS Full-text

DOCUMENT NUMBER: 136:230299

TITLE: Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver

AUTHOR(S): Xu, Xiang-Ru; Huang, Jian; Xu, Zhi-Gang; Qian, Bin-Zhi; Zhu, Zhi-Dong; Yan, Qing; Cai, Ting; Zhang, Xin; Xiao, Hua-Sheng; Qu, Jian; Liu, Feng; Huang, Qiu-Hua; Cheng, Zhi-Hong; Li, Neng-Gan; Du, Jian-Jun; Hu, Wei; Shen, Kun-Tang; Lu, Gang; Fu, Gang; Zhong, Ming; Xu, Shu-Hua; Gu, Wen-Yi; Huang, Wei; Zhao, Xin-Tai; Hu, Geng-Xi; Gu, Jian-Ren; Chen, Zhu; Han, Ze-Guang

CORPORATE SOURCE: Chinese National Human Genome Center at Shanghai, Shanghai, 201203, Peop. Rep. China

SOURCE: Proceedings of the National Academy of Sciences of the

United States of America (2001), 98(26), 15089-15094  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Human hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. In this work, the authors report on a comprehensive characterization of gene expression profiles of hepatitis B virus-pos. HCC through the generation of a large set of 5'-read expressed sequence tag (EST) clusters (11,065 in total) from HCC and noncancerous liver samples, which then were applied to a cDNA microarray system containing 12,393 genes/ESTs and to comparison with a public database. The com. cDNA microarray, which contains 1,176 known genes related to oncogenesis, was used also for profiling gene expression. Integrated data from the above approaches identified 2,253 genes/ESTs as candidates with differential expression. A number of genes related to oncogenesis and hepatic function/differentiation were selected for further semiquant. reverse transcriptase-PCR anal. in 29 paired HCC/noncancerous liver samples. Many genes involved in cell cycle regulation such as cyclins, cyclin-dependent kinases, and cell cycle neg. regulators were deregulated in most patients with HCC. Aberrant expression of the Wnt- $\beta$ -catenin pathway and enzymes for DNA replication also could contribute to the pathogenesis of HCC. The alteration of transcription levels was noted in a large number of genes implicated in metabolism, whereas a profile change of others might represent a status of dedifferentiation of the malignant hepatocytes, both considered as potential markers of diagnostic value. Notably, the altered transcriptome profiles in HCC could be correlated to a number of chromosome regions with amplification or loss of heterozygosity, providing one of the underlying causes of the transcription anomaly of HCC.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 12 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN DUPLICATE 3

ACCESSION NUMBER: 2001:355366 BIOSIS Full-text  
DOCUMENT NUMBER: PREV200100355366  
TITLE: Pharmacophore and three-dimensional quantitative structure activity relationship methods for modeling cytochrome P450 active sites.  
AUTHOR(S): Ekins, Sean [Reprint author]; de Groot, Marcel J.; Jones, Jeffrey P.  
CORPORATE SOURCE: Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN, 46285, USA  
ekins\_sean@lilly.com  
SOURCE: Drug Metabolism and Disposition, (July, 2001) Vol. 29, No. 7, pp. 936-944. print.  
CODEN: DMSDAI. ISSN: 0090-9556.  
DOCUMENT TYPE: Article  
General Review; (Literature Review)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 2 Aug 2001  
Last Updated on STN: 19 Feb 2002

AB Structure activity relationships (SAR), three-dimensional structure activity relationships (3D-QSAR), and pharmacophores represent useful tools in understanding cytochrome P450 (CYP) active sites in the absence of crystal structures for these human enzymes. These approaches have developed over the last 30 years such that they are now being applied in numerous industrial and academic laboratories solely for this purpose. Such computational approaches have helped in understanding substrate and inhibitor binding to the major human CYPs 1A2, 2B6, 2C9, 2D6, 3A4 as well as other CYPs and additionally complement homology models for these enzymes. Similarly, these approaches may assist in our understanding of CYP induction. This review describes in detail the development of pharmacophores and 3D-QSAR techniques, which are now being more widely used for modeling CYPs; the review will also describe how such approaches are likely to further impact our active site knowledge of these omnipresent and important enzymes.

L16 ANSWER 13 OF 32 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2001636239 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 11688945  
TITLE: A virtual high throughput screen for high affinity cytochrome P450cam substrates. Implications for in silico prediction of drug metabolism.  
AUTHOR: Keseru G M  
CORPORATE SOURCE: Computer Assisted Drug Discovery, Gedeon Richter Ltd., Budapest, Hungary.. gy.keseru@richter.hu

SOURCE: Journal of computer-aided molecular design, (2001 Jul) 15  
(7) 649-57.

Journal code: 8710425. ISSN: 0920-654X.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200204

ENTRY DATE: Entered STN: 20011105

Last Updated on STN: 20020409

Entered Medline: 20020408

AB Structure-based virtual screening techniques require reliable scoring functions to discriminate potential substrates effectively. In this study we compared the performance of GOLD, PMF, DOCK and FlexX scoring functions in FlexX flexible docking to cytochrome P450cam binding site. Crystal structures of protein-substrate complexes were most effectively reproduced by the FlexX/PMF method. On the other hand, the FlexX/GOLD approach provided the best correlation between experimental binding constants and predicted scores. Binding modes selected by the FlexX/PMF approach were rescored by GOLD to obtain a reliable measure of binding energetics. The effectiveness of the FlexX/PMF/GOLD method was demonstrated by the correct classification of 32 out of the 33 experimentally studied compounds and also in a virtual HTS test on a library of 10,000 compounds. Although almost all the available functions were developed to be general, our study on cytochrome P450cam substrates suggests that careful selection or even tailoring the scoring function might increase the prediction power of virtual screens significantly. The FlexX/PMF/GOLD methodology was tested on cytochrome P450 3A4 substrates and inhibitors. This preliminary study revealed that the combined function was able to recognise 334 out of the 345 compounds bound to 3A4.

L16 ANSWER 14 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2001311394 EMBASE Full-text

TITLE: The PXR ligand-binding domain: How to be picky and promiscuous at the same time.

AUTHOR: Gillan E.M.J.

CORPORATE SOURCE: gillam@plpk.uq.edu.au

SOURCE: Trends in Pharmacological Sciences, (1 Sep 2001) Vol. 22,  
No. 9, pp. 448.

Refs: 1

ISSN: 0165-6147 CODEN: TPHSDY

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Note

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

ENTRY DATE: Entered STN: 20010920

Last Updated on STN: 20010920

DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L16 ANSWER 15 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2001:228049 BIOSIS Full-text

DOCUMENT NUMBER: PREV200100228049

TITLE: Prediction of midazolam-CYP3A inhibitors interaction in the human liver from in vivo/in vitro absorption, distribution, and metabolism data.

AUTHOR(S): Yamano, Katsuhiro [Reprint author]; Yamamoto, Koujiro;  
Katashima, Masataka; Kotaki, Hajime; Takedomi, Sayuri;  
Matsuo, Hirotsami; Ohtani, Hisakazu; Sawada, Yasufumi; Iga,  
Tatsuji

CORPORATE SOURCE: Biopharmaceutical and Pharmacokinetic Research  
Laboratories, Fujisawa Pharmaceutical Co., Ltd., 1-6,  
Kashima 2-chome, Yodogawa-ku, Osaka, 532-8514, Japan  
katsuhiro\_yamano@po.fujisawa.co.jp

SOURCE: Drug Metabolism and Disposition, (April, 2001) Vol. 29, No.  
4 Part 1, pp. 443-452. print.

CODEN: DMDSAI. ISSN: 0090-9556.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 9 May 2001

Last Updated on STN: 19 Feb 2002



AB The extent of decreases in apparent hepatic clearance and intrinsic hepatic clearance of midazolam (MDZ) after intravenous administration of MDZ with concomitant oral administration of cimetidine (CIM), itraconazole (ITZ), or erythromycin (EM) was predicted using plasma unbound concentrations and liver unbound concentrations of inhibitors. When MDZ was concomitantly administered with CIM, the observed increase in MDZ concentration was successfully predicted using inhibition constants assessed by human liver microsome and liver-to-plasma unbound concentration ratios in rats. However, the extent of interaction with ITZ or EM was still underestimated even taking into account the concentrative uptake of inhibitors into liver. We could predict the degree of "mechanism-based" inhibition by EM on the hepatic metabolism of MDZ, after repeated administration of EM, by a physiological model incorporating the amount of active enzyme as well as the concentration of inhibitor. The maximum inactivation rate constant and the apparent inactivation constant of EM on MDZ metabolism were 0.0665 min<sup>-1</sup> and 81.8 μM, respectively. These kinetic parameters for the inactivation of the enzyme were applied to the physiological model with pharmacokinetic parameters of EM and MDZ obtained from published results. Consequently, we estimated that cytochrome P450 3A4 in the liver after repeated oral administration of EM was inactivated, resulting in 2.6-fold increase in the plasma concentration of MDZ. The estimated extent of increase in MDZ concentration in our study correlated well with the observed value based on metabolic inhibition by EM from published results.

L16 ANSWER 16 OF 32 MEDLINE on STN DUPLICATE 5  
 ACCESSION NUMBER: 2001698384 MEDLINE Full-text  
 DOCUMENT NUMBER: PubMed ID: 11744061  
 TITLE: Apo-cytochrome b5 as an indicator of changes in heme accessibility: preliminary studies with cytochrome P450 3A4.  
 AUTHOR: Gilep A A; Guryev O L; Usanov S A; Estabrook R W  
 CORPORATE SOURCE: Department of Biochemistry, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9038, USA.  
 CONTRACT NUMBER: GM16488-32 (NIGMS)  
 SOURCE: Journal of inorganic biochemistry, (2001 Dec 15) 87 (4) 237-44.  
 Journal code: 7905788. ISSN: 0162-0134.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200201  
 ENTRY DATE: Entered STN: 20011218  
 Last Updated on STN: 20020125  
 Entered Medline: 20020114

AB Cytochrome P450s (P450 or CYP) are the largest family of hemeproteins yet characterized. X-ray crystallographic studies have shown that the heme of the P450 hemeproteins is buried in the interior of the protein molecule. Unexplored are answers to questions concerning the role of heme in the folding of newly synthesized apo-P450s and the factors that influence changes in heme accessibility following modification of the pattern of folding of the holo-P450s. We have carried out the present studies to measure changes in heme accessibility in P450s. This is an initial step to determining whether heme-binding confers structural and functional integrity and stability to a P450 molecule. Recently, we have shown that apo-high molecular weight cytochrome b5 (apo-HMWb5) is an efficient acceptor of heme when added to a preparation of purified recombinant CYP3A4. In the present work we have studied heme binding by apo-HMWb5 when mixed with a number of different hemeproteins (myoglobin, hemoglobin, catalase, CYP4A1, CYP101, and CYP3A4). These hemeproteins differ in the location of the heme (i.e., surface or internal) allowing one to study changes in structure as measured by the process of heme transfer from one protein to another. It was found that heme transfer to apo-HMWb5 occurs relatively rapidly from hemeproteins where the heme is located at or near the surface or when the hemeprotein is denatured. In contrast, heme transfer from P450s to apo-HMWb5 occurs only following modification of the P450 structure with chaotropic agents. An exception is CYP3A4 where a measurable amount of heme is transferred to apo-HMWb5 in the absence of denaturing agents. The preliminary results described here employs apo-HMWb5 as an indicator for assessing changes in heme-availability of P450s as the protein-folding of the molecule is altered.

L16 ANSWER 17 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 ACCESSION NUMBER: 2001:482335 BIOSIS Full-text

DOCUMENT NUMBER: PREV200100482335  
TITLE: Analysis of covalently modified cytochrome P450s by mass spectrometry.  
AUTHOR(S): Lightning, Luke Koenigs [Reprint author]; Trager, William F.  
CORPORATE SOURCE: Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, RHPH Room 406C, West Lafayette, IN, 47907, USA  
lukelightning@yahoo.com  
SOURCE: Abstracts of Papers American Chemical Society, (2001) Vol. 222, No. 1-2, pp. TOXI 6. print.  
Meeting Info.: 222nd National Meeting of the American Chemical Society. Chicago, Illinois, USA. August 26-30, 2001. American Chemical Society.  
CODEN: ACSRAL. ISSN: 0065-7727.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 17 Oct 2001  
Last Updated on STN: 23 Feb 2002

AB Elucidation of the key structural elements responsible for cytochrome P450 activity is an area of considerable importance to the field of drug metabolism. Because crystal structure data for human P450s remains elusive, active site information can only be obtained using alternative methods. One method relies on the use of compounds that are oxidized to reactive species that covalently modify the P450 at the active site. The site of modification and, hence, the identity of active site residues can be determined using various mass spectrometric techniques. Cytochromes P450 2A6, 2B1, 2C9, and 3A4 were expressed, purified, and subjected to mechanism-based inactivation by various compounds. As determined by HPLC/radiometric detection, mechanism-based inactivation resulted in the covalent attachment of the drug to the P450. ESI- and MALDI-MS analysis of the intact and digested proteins, respectively, were used to determine the number of molecules that were attached, the pathway of inactivation, and the site of modification.

L16 ANSWER 18 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:306248 SCISEARCH Full-text  
THE GENUINE ARTICLE: 304LB  
TITLE: Mechanism-based inactivation of cytochrome P450 3A4 by L-754,394  
AUTHOR: Lightning L K; Jones J P; Friedberg T; Pritchard M P; Shou M; Rushmore T H; Trager W F (Reprint)  
CORPORATE SOURCE: Univ Washington, Dept Med Chem, Box 357510, Seattle, WA 98195 USA (Reprint); Univ Washington, Dept Med Chem, Seattle, WA 98195 USA; Washington State Univ, Dept Chem, Pullman, WA 99164 USA; Univ Dundee, Ninewells Hosp & Med Sch, Biomed Res Ctr, Dundee DD1 9SY, Scotland; Merck Res Labs, Dept Drug Metab, W Point, PA 19486 USA  
COUNTRY OF AUTHOR: USA; Scotland  
SOURCE: BIOCHEMISTRY, (18 APR 2000) Vol. 39, No. 15, pp. 4276-4287  
ISSN: 0006-2960.  
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 50  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Mechanism-based inactivation of human liver P450 3A4 by L-754,394, a Merck compound synthesized as a potential HIV protease inhibitor, was investigated using recombinant P350 3A4. Enzyme inactivation was characterized by a small partition ratio (3.3 or 4.3 +/- 0.4), i.e., the total number of metabolic events undergone by the inhibitor divided by the number of enzyme inactivating events, lack of reversibility upon extensive dialysis, no decrease in the characteristic 450-nm species relative to control, and covalent modification of the apoprotein. The major and minor products formed during the inactivation of P450 3A4 were the monohydroxylated and the dihydrodiol metabolites of L-754,394, respectively. L-754,394 that had been adducted to P350 3A4 was hydrolyzed under the conditions used for SDS-PAGE, Ni2+ affinity chromatography, and proteolytic digestion. In addition, the modification was not

stable to the acidic conditions of HPLC separation and CNBr digestion. The labile nature of the peptide adduct and the nonstoichiometric binding of the inactivating species to P350 3A4 precluded the direct identification of a covalently modified amino acid residue or the peptide to which it was attached. However, Tricine SDS-PAGE in combination with MALDI-TOF-MS and homology modeling, allowed I257-M317 to be tentatively identified as an active site peptide, while prior knowledge of the stability of N-, O-, and S-linked conjugates of activated furans implicates Glu307 as the active site amino acid that is labeled by L-754, 394.

L16 ANSWER 19 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:266561 SCISEARCH Full-text  
 THE GENUINE ARTICLE: 299JK  
 TITLE: Crystal structures of ligand complexes of P450eryF exhibiting homotropic cooperativity  
 AUTHOR: Cupp-Vickery J (Reprint); Anderson R; Hatziris Z  
 CORPORATE SOURCE: Calif State Univ Fullerton, Dept Chem & Biochem, 800 N State Coll Blvd, Fullerton, CA 92834 USA (Reprint); Calif State Univ Fullerton, Dept Chem & Biochem, Fullerton, CA 92834 USA  
 COUNTRY OF AUTHOR: USA  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (28 MAR 2000) Vol. 97, No. 7, pp. 3050-3055.  
 ISSN: 0027-8424.  
 PUBLISHER: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 29  
 ENTRY DATE: Entered STN: 2000  
 Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Several mammalian cytochrome P450 (P450) isoforms demonstrate homotropic cooperativity with a number of substrates, including steroids and polycyclic aromatic hydrocarbons. To identify structural factors contributing to steroid and polycyclic aromatic hydrocarbon binding to P450 enzymes and to determine the location of the allosteric site, we investigated interactions of the macrolide hydroxylase P450eryF from *Saccharopolyspora erythraea* with androstenedione and 9-aminophenanthrene. Spectroscopic binding assays indicate that P450eryF binds androstenedione with an affinity of 365  $\mu$ M and a Hill coefficient of 1.31  $\pm$  0.6 and coordinates with 9-aminophenanthrene with an affinity of 91  $\mu$ M and a Hill coefficient of 1.38  $\pm$  0.2. Crystals of complexes of androstenedione and 9-aminophenanthrene with P450eryF were grown and diffracted to 2.1 Angstrom and 2.35 Angstrom, respectively. Electron density maps indicate that for both complexes two ligand molecules are simultaneously present in the active site. The P450eryF/ androstenedione model was refined to an  $r$  = 18.9%, and the two androstenedione molecules have similar conformations. The proximal androstenedione is positioned such that the alpha-face of carbon-6 is closest to the heme iron, and the second steroid molecule is positioned 5.5 Angstrom distal in the active site. The P450eryF/9-aminophenanthrene model was refined to an  $r$  = 19.7% with the proximal 9-aminophenanthrene coordinated with the heme iron through the 9-amino group and the second ligand positioned approximate to 6 Angstrom distal in the active site. These results establish that homotropic cooperativity in ligand binding can result from binding of two substrate molecules within the active site pocket without major conformational changes in the protein.

L16 ANSWER 20 OF 32 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 2000161938 MEDLINE Full-text  
 DOCUMENT NUMBER: PubMed ID: 10698112  
 TITLE: Cytochrome P450 catalyzed nitric oxide synthesis: a theoretical study.  
 AUTHOR: Keseru G M; Volk B; Balogh G T  
 CORPORATE SOURCE: Department of Chemical Information Technology, Technical University of Budapest, Hungary.. gy.keseru@richter.hu  
 SOURCE: Journal of biomolecular structure & dynamics, (2000 Feb) 17 (4) 759-67.  
 Journal code: 8404176. ISSN: 0739-1102.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200004  
ENTRY DATE: Entered STN: 20000421  
Last Updated on STN: 20000421  
Entered Medline: 20000411

AB Similar to nitric oxide synthase (NOS) cytochrome P450 isoforms (e.g. 3A and 4E) can produce nitric oxide from arginine. Although the active site of both proteins contains a protoporphyrin IX unit having an axial cystein ligand, their effectiveness in the synthesis of NO differs significantly. Now the molecular basis of this functional difference was investigated. A homology model for cytochrome P450 3A4 was refined and compared to the X-ray structure of iNOS. We found the active site of iNOS to be more readily accessible for the substrate than that of P450. Docking calculations were performed using the Monte Carlo conformational analysis technique on all internal and external degrees of freedom of arginine and active site residues as well. The lowest energy conformation of the cytochrome P450 3A4-substrate complex was compared to the high resolution X-ray structure of the iNOS-arginine complex. Comparison of substrate orientations revealed that arginine binds in a similar conformation in both enzymes. In contrast to iNOS we found, however, that in P450 partially negative propionate side chains of protoporphyrin IX are located on the opposite side of the heme plane. As a result of this and the absence of other negatively charged residues the distal (substrate binding) side of P450 should be less negative than that of NOS and therefore its affinity toward the partially positive arginine is reduced. Comparison of molecular electrostatic potentials calculated within the active site of the proteins supports this proposal. Reduced affinity in combination with limited substrate access might be responsible for the less effective NO synthesis of cytochrome P450 observed experimentally.

L16 ANSWER 21 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2000:393619 SCISEARCH Full-text  
THE GENUINE ARTICLE: 317FY  
TITLE: Dual protease inhibitor therapy in HIV-infected patients:  
Pharmacologic rationale and clinical benefits  
AUTHOR: Flexner C (Reprint)  
CORPORATE SOURCE: Johns Hopkins Univ, Sch Med, Dept Med & Pharmacol, Div  
Clin Pharmacol, Baltimore, MD 21287 USA (Reprint); Johns  
Hopkins Univ, Sch Med, Dept Mol Sci, Baltimore, MD 21287  
USA  
COUNTRY OF AUTHOR: USA  
SOURCE: ANNUAL REVIEW OF PHARMACOLOGY AND TOXICOLOGY, (2000) Vol.  
40, pp. 649-674.  
ISSN: 0362-1642.  
PUBLISHER: ANNUAL REVIEWS, 4139 EL CAMINO WAY, PO BOX 10139, PALO  
ALTO, CA 94303-0139 USA.  
DOCUMENT TYPE: General Review; Journal  
LANGUAGE: English  
REFERENCE COUNT: 84  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB HIV protease inhibitors, as components of combination antiretroviral drug regimens, have substantially reduced the morbidity and mortality associated with HIV infection. They selectively block the action of the virus-encoded protease and stop the virus from replicating. In general, these drugs have poor systemic bioavailability and must be dosed with respect to meals for optimal absorption. Protease inhibitor-containing regimens require ingestion of a large number of capsules, are costly, and produce or are susceptible to metabolic drug interactions. Simultaneous administration of two protease inhibitors takes advantage of beneficial pharmacokinetic interactions and may circumvent many of the drugs' undesirable pharmacologic properties. For example, ritonavir increases saquinavir concentrations at steady state by up to 30-fold, allowing reduction of saquinavir dose and dosing frequency. Ritonavir decreases the systemic clearance of indinavir and overcomes the deleterious effect of food on indinavir bioavailability. These benefits reflect inhibition of presystemic clearance and first-pass metabolism, as well as inhibition of systemic clearance mediated by hepatic cytochrome P450 3A4. Several dual protease inhibitor combination regimens have shown great promise in clinical trials and are now recommended as components of salvage therapy for HIV-infected patients.

L16 ANSWER 22 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.

on STN

ACCESSION NUMBER: 2001020792 EMBASE Full-text  
TITLE: Modelling human cytochromes P450 for evaluating drug metabolism: An update.  
AUTHOR: Lewis D.F.V.  
CORPORATE SOURCE: D.F.V. Lewis, Sch. of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey GU2 7XH, United Kingdom. d.lewis@surrey.ac.uk  
SOURCE: Drug Metabolism and Drug Interactions, (2000) Vol. 16, No. 4, pp. 307-324.  
Refs: 29  
ISSN: 0792-5077 CODEN: DMDIEQ  
COUNTRY: Israel  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
030 Pharmacology  
037 Drug Literature Index  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20010208  
Last Updated on STN: 20010208

AB Cytochrome P450 (CYP) enzymes represent the major catalysts for the Phase 1 metabolism of drugs and other xenobiotics in Mammalia, including Homo sapiens. There is considerable current interest in evaluating and, consequently, predicting the metabolic fate of new chemical entities (NCEs) via modelling molecular interactions with P450 constructs, such that sites of metabolism, particular CYP involvement and binding affinities, can be estimated. This paper focuses on the principles for homology modelling of typical enzyme-substrate interactions within the putative active sites of major P450s associated with drug metabolism in man. It also represents an update on previously published work in this journal/1/.

L16 ANSWER 23 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:143641 SCISEARCH Full-text  
THE GENUINE ARTICLE: 285GT  
TITLE: The importance of SRS-1 residues in catalytic specificity of human cytochrome p450 3A4  
AUTHOR: Roussel F (Reprint); Khan K K; Halpert J R  
CORPORATE SOURCE: Univ Texas, Med Branch, Dept Pharmacol & Toxicol, 301 Univ Blvd, Galveston, TX 77555 USA (Reprint); Univ Texas, Med Branch, Dept Pharmacol & Toxicol, Galveston, TX 77555 USA  
COUNTRY OF AUTHOR.: USA  
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (15 FEB 2000) Vol. 374, No. 2, pp. 269-278.  
ISSN: 0003-9861.  
PUBLISHER: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 55  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The structural basis for the regioselective hydroxylation of Delta-4-3-ketosteroids by human CYP3A4 was investigated. Prior studies had suggested that the chemical reactivity of the allylic 6 beta-position might have a greater influence than steric constraints by the enzyme. Six highly conserved CYP3A residues from substrate recognition site 1 were examined by site-directed mutagenesis. F102A and A117L showed no spectrally detectable P450. V101G and T103A exhibited a wild-type progesterone metabolite profile. Of five mutants at residue N104, only N104D yielded holoenzyme and exhibited the same steroid metabolite profile as wild-type. Of four mutants at position S119 (A, L, T, V), the three hydrophobic ones produced 2 beta-OH rather than 6 beta-OH progesterone or testosterone as the major metabolite. Kinetic analysis showed S-50 values similar to wild-type for S119A (progesterone) and S119V (testosterone), whereas the V-max values for 2 beta-hydroxysteroid formation were increased in both cases. All four mutants exhibited an altered product profile for 7-hexoxycoumarin side-chain hydroxylation, whereas the stimulation of steroid hydroxylation by alpha-naphthoflavone was similar to the wild-type. The results indicate that the highly conserved residue S119 is a key determinant of CYP3A4

specificity and reveal an important role of the active site topology in steroid 6 beta-hydroxylation. (C) 2000 Academic Press.

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on STN

ACCESSION NUMBER: 2000070429 EMBASE Full-text  
TITLE: Theoretical investigation of substrate specificity for cytochromes P450 IA2, P450 IID6 and P450 IIIA4.  
AUTHOR: De Rienzo F.; Fanelli F.; Menziani M.C.; De Benedetti P.G.  
CORPORATE SOURCE: P.G. De Benedetti, Dipartimento di Chimica, Univ. di Modena e Reggio Emilia, Via Campi 183, I-41100 Modena, Italy. deben@unino.it  
SOURCE: Journal of Computer-Aided Molecular Design, (2000) Vol. 14, No. 1, pp. 93-116.  
Refs: 65  
ISSN: 0920-654X CODEN: JCADEQ  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20000309  
Last Updated on STN: 20000309

AB Three-dimensional models of the cytochromes P450 IA2, P450 IID6 and P450 IIIA4 were built by means of comparative modeling using the X-ray crystallographic structures of P450 CAM, P450 BM-3, P450 TERP and P450 ERYF as templates. The three cytochromes were analyzed both in their intrinsic structural features and in their interaction properties with fifty specific and non-specific substrates. Substrate/enzyme complexes were obtained by means of both automated rigid and flexible body docking. The comparative analysis of the three cytochromes and the selected substrates, in their free and bound forms, allowed for the building of semi-quantitative models of substrate specificity based on both molecular and intermolecular interaction descriptors. The results of this study provide new insights into the molecular determinants of substrate specificity for the three different eukaryotic P450 isozymes and constitute a useful tool for predicting the specificity of new compounds.

L16 ANSWER 25 OF 32 MEDLINE on STN

ACCESSION NUMBER: 1999340158 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 10411567  
TITLE: Use of the steroid derivative RPR 106541 in combination with site-directed mutagenesis for enhanced cytochrome P-450 3A4 structure/function analysis.  
AUTHOR: Stevens J C; Domanski T L; Harlow G R; White R B; Orton E; Halpert J R  
CORPORATE SOURCE: Department of Drug Metabolism and Pharmacokinetics, Rhone-Poulenc Rorer, Collegeville, Pennsylvania, USA.. Jeffrey.STEVENSON@RP-Rorer  
CONTRACT NUMBER: GM19058 (NIGMS)  
GM54995 (NIGMS)  
SOURCE: Journal of pharmacology and experimental therapeutics, (1999 Aug) 290 (2) 594-602.  
Journal code: 0376362. ISSN: 0022-3565.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199908  
ENTRY DATE: Entered STN: 19990827  
Last Updated on STN: 19990827  
Entered Medline: 19990818

AB RPR 106541 (20R-16alpha,17alpha-[butylidenebis(oxy)]-6al pha, 9alpha-difluoro-11beta-hydroxy-17beta-(methylthio)androst a-4-en-3-one) is an airway-selective steroid developed for the treatment of asthma. Two metabolites produced by human liver microsomes were identified as R- and S-sulfoxide diastereomers based on liquid chromatography/mass spectrometry analysis, proton nuclear magnetic resonance, and cochromatography with standards. Sulfoxide formation was determined to be cytochrome P-450 (CYP) 3A4-dependent by correlation with CYP3A4-marker nifedipine oxidase activity, inhibition by cyclosporin A and troleandomycin, and inhibition of R- (70%) and S- (64%) sulfoxide formation by anti-3A antibody. Expressed CYP2C forms catalyzed RPR 106541 sulfoxidation; however, other

phenotyping approaches failed to confirm the involvement of CYP2C forms in these reactions in human liver microsomes. Expressed CYP3A4 catalyzed the formation of the sulfoxide diastereomers in a 1:1 ratio, whereas CYP3A5 displayed stereoselectivity for formation of the S-diastereomer. The high rate of sulfoxidation by CYP3A4 and the blockage of oxidative metabolism at the electronically favored 6beta-position provided advantages for RPR 106541 over other substrates as an active site probe of CYP3A4. Therefore, oxidation of RPR 106541 by various CYP3A4 substrate recognition site (SRS) mutants was assessed. In SRS-4, A305V and F304A showed dramatically reduced rates of R-diastereomer formation (83 and 64% decreases, respectively), but S-diastereomer formation was affected to a lesser extent. A370V (SRS-5) showed decreased formation of the R-sulfoxide (52%) but increased formation of the S-diastereomer. In the SRS-2 region, the most dramatic change in sulfoxide ratios was observed for L210A. In conclusion, the structure of RPR 106541 imposes specific constraints on enzyme binding and activity and thus represents an improved CYP3A4 probe substrate.

L16 ANSWER 26 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 1999:431467 BIOSIS Full-text  
DOCUMENT NUMBER: PREV199900431467  
TITLE: Homology modelling of human cytochromes P450 involved in xenobiotic metabolism and rationalization of substrate selectivity.  
AUTHOR(S): Lewis, David F. V. [Reprint author]  
CORPORATE SOURCE: School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK  
SOURCE: Experimental and Toxicologic Pathology, (July, 1999) Vol. 51, No. 4-5, pp. 369-374. print.  
CODEN: ETPAEK. ISSN: 0940-2993.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 18 Oct 1999  
Last Updated on STN: 18 Oct 1999

AB Molecular modelling of human cytochrome P450 (CYP) isoforms is described, based on amino acid sequence homology with a unique bacterial P450 (CYP102) of known crystal structure. It is found that for the human hepatic P450s involved in the metabolism of xenobiotics, ie. CYP1A2, CYP1A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, there is a satisfactory agreement between specific substrate characteristics and topographical features of the putative active sites, including complementarity with key amino acid residues in the P450 haem environments. A combination of homology model interactions with substrates and certain molecular properties of the compounds themselves provides a methodology for the evaluation of potential P450 selectivity in new chemical entities (NCEs).

L16 ANSWER 27 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 1999:4895 SCISEARCH Full-text  
THE GENUINE ARTICLE: 150WV  
TITLE: Molecular basis of P450 inhibition and activation - Implications for drug development and drug therapy  
AUTHOR: Szklarz G D; Halpert J R (Reprint)  
CORPORATE SOURCE: Univ Texas, Med Branch, Dept Pharmacol & Toxicol, 301 Univ Blvd, Galveston, TX 77555 USA (Reprint); Univ Texas, Med Branch, Dept Pharmacol & Toxicol, Galveston, TX 77555 USA; W Virginia Univ, Dept Basic Pharmaceut Sci, Morgantown, WV 26506 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: DRUG METABOLISM AND DISPOSITION, (DEC 1998) Vol. 26, No. 12, pp. 1179-1184.  
ISSN: 0090-9556.  
PUBLISHER: AMER SOC PHARMACOLOGY EXPERIMENTAL THERAPEUTICS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 25  
ENTRY DATE: Entered STN: 1999  
Last Updated on STN: 1999  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Three-dimensional homology models of cytochromes P450 (P450) 2B1 and P450 3A4 have been utilized along with site-directed mutagenesis to elucidate the molecular

determinants of substrate specificity. Most of the key residues identified in 28 enzymes fall within five substrate recognition sites (SRSs) and have counterparts in bacterial P450 residues that regulate substrate binding or access. Docking of inhibitors into 28 models has provided a plausible explanation for changes in susceptibility to mechanism-based inactivation that accompany particular amino acid side-chain replacements. These studies provide a basis for predicting drug interactions due to P450 inhibition and for rational inhibitor design. In addition, the location of P450 3A4 residues capable of influencing homotropic stimulation by substrates and heterotropic stimulation by flavonoids has been identified. Steroid hydroxylation by the wild-type enzyme exhibits sigmoidal kinetics, indicative of positive cooperativity. Based on the 3A4 model and single-site mutants, a double mutant in SRS-P has been constructed that exhibits normal Michaelis-Menten kinetics. Results of modeling and mutagenesis studies suggest that the substrate and effector bind at adjacent sites within a single large cavity in P450 3A4. A thorough understanding of the location and structural requirements of the substrate-binding and effector sites in cytochrome P450 3A4 should prove valuable in rationalizing and predicting interactions among the multitude of drugs and other compounds that bind to the enzyme.

L16 ANSWER 28 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 1998:202510 SCISEARCH Full-text  
THE GENUINE ARTICLE: ZB037  
TITLE: Analysis of four residues within substrate recognition  
site 4 of human cytochrome p450 3A4:  
Role in steroid hydroxylase activity and  
alpha-naphthoflavone stimulation  
AUTHOR: Domanski T L (Reprint); Liu J P; Harlow G R; Halpert J R  
CORPORATE SOURCE: Univ Arizona, Coll Pharm, Dept Pharmacol & Toxicol,  
Tucson, AZ 85721 USA (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (15 FEB 1998)  
Vol. 350, No. 2, pp. 223-232.  
ISSN: 0003-9861.  
PUBLISHER: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA  
92101-4495 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 44  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Sequence alignment of human cytochrome P450 3A4 with bacterial enzymes of known structure has provided a basis from which to predict residues involved in substrate oxidation. Substitutions were made at four residues (I301, F304, A305, and T309) predicted to be located within the highly conserved substrate recognition site 4. Site-directed mutants engineered to contain carboxy-terminal histidine tags were expressed in *Escherichia coli* and purified on a metal affinity column. The integrity of each protein was assessed by SDS-polyacrylamide gel electrophoresis and immunoblotting. Functional analysis was performed using progesterone and testosterone as substrates and alpha-naphthoflavone as an activator. In testosterone hydroxylase assays, all of the mutants displayed rates of total product formation similar to wild-type 3A4, with several mutants showing small differences in specific products formed. However, with progesterone as the substrate, mutants F304A, A305V, and T309A exhibited altered product ratios and/or changes in the rates of product formation. F304A and A305V also displayed altered flavonoid stimulation that resulted in product ratios dramatically different from wild-type 3A4. Therefore, the kinetics of progesterone hydroxylation of these mutants and the wildtype enzyme were further assessed, and the data were analyzed with the Hill equation. Results with wildtype 3A4 and F304A indicated that at high progesterone concentrations, hydroxylation rates and product ratios are independent of the presence of alpha-NF. This suggests that progesterone may be equivalent to alpha-NF as an activator. In contrast, A305V exhibited autoactivation by progesterone but inhibition by alpha-NF. (C) 1998 Academic Press.

L16 ANSWER 29 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 1997:562477 SCISEARCH Full-text  
THE GENUINE ARTICLE: XM538  
TITLE: Identification of three key residues in substrate



recognition site 5 of human cytochrome P450  
3A4 by cassette and site-directed mutagenesis  
AUTHOR: He Y A (Reprint); He Y Q; Szklarz G D; Halpert J R  
CORPORATE SOURCE: UNIV ARIZONA, COLL PHARM, DEPT PHARMACOL & TOXICOL,  
TUCSON, AZ 85721  
COUNTRY OF AUTHOR: USA  
SOURCE: BIOCHEMISTRY, (22 JUL 1997) Vol. 36, No. 29, pp. 8831-8839

ISSN: 0006-2960.  
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 46  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Cassette mutagenesis and site-directed mutagenesis were used to investigate the importance of individual amino acid residues at positions 364-377 of cytochrome P450 3A4 in determining steroid hydroxylation or stimulation by alpha-naphthoflavone. The mutants were expressed in an Escherichia coli system, and solubilized membranes were prepared. All mutants except R365G and R365K exhibited anti-3A immunoreactivity on Western blotting, although R372S and R375K were not detected as the Fe2+-CO complex. Replacement of Arg-372 by Lys yielded a typical P450 spectrum. The results indicate that the highly conserved Arg residues at positions 365 and 375 may play a role in stabilizing the tertiary structure or in heme binding. Catalytic activities of 12 mutants were examined using progesterone and testosterone as substrates, and residues 369, 370, and 373 were found to play an important role in determining substrate specificity. Although the three mutants hydroxylated progesterone and testosterone primarily at the 6 beta-position like the wild-type, replacement of Ile-369 by Val suppressed progesterone 16 alpha-hydroxylase activity, whereas substitution of Ala-370 with Val enhanced progesterone 16 alpha-hydroxylation. Interestingly, substitution of Leu-373 with His resulted in production of a new metabolite from both steroids. Moreover, the mutants at positions 369 and 373 were more and less responsive, respectively, than the wild-type to alpha-naphthoflavone one stimulation. Alterations in activities or expression of several mutants were interpreted using a three-dimensional model of P450 3A4. The results suggest that analogy with mammalian family 2 and bacterial cytochromes P450 can be used to predict P450 3A residues that contribute to regiospecific steroid hydroxylation.

L16 ANSWER 30 OF 32 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 97409492 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 9263853  
TITLE: Molecular modeling of cytochrome P450 3A4

AUTHOR: Szklarz G D; Halpert J R  
CORPORATE SOURCE: Department of Pharmacology and Toxicology, College of  
Pharmacy, University of Arizona, Tucson 85721, USA..  
Szklarz@tonic.pharm.arizona.edu  
CONTRACT NUMBER: ES03619 (NIEHS)  
ES06694 (NIEHS)  
SOURCE: Journal of computer-aided molecular design, (1997 May) 11  
(3) 265-72.  
Journal code: 8710425. ISSN: 0920-654X.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: SWISSPROT-P08684  
ENTRY MONTH: 199710  
ENTRY DATE: Entered STN: 19971021  
Last Updated on STN: 19971021  
Entered Medline: 19971007

AB The three-dimensional structure of human cytochrome P450 3A4 was modeled based on crystallographic coordinates of four bacterial P450s; P450 BM-3, P450cam, P450terp, and P450eryF. The P450 3A4 sequence was aligned to those of the known proteins using a structure-based alignment of P450 BM-3, P450cam, P450terp, and P450eryF. The coordinates of the model were then calculated using a consensus strategy, and the final structure was optimized in the presence of water. The P450 3A4 model resembles P450 BM-3 the most, but the B' helix is similar to that of P450eryF, which leads to an enlarged active site when

compared with P450 BM-3, P450cam, and P450terp. The 3A4 residues equivalent to known substrate contact residues of the bacterial proteins and key residues of rat P450 2B1 are located in the active site or the substrate access channel. Docking of progesterone into the P450 3A4 model demonstrated that the substrate bound in a 6 beta-orientation can interact with a number of active site residues, such as 114, 119, 301, 304, 305, 309, 370, 373, and 479, through hydrophobic interactions. The active site of the enzyme can also accommodate erythromycin, which, in addition to the residues listed for progesterone, also contacts residues 101, 104, 105, 214, 215, 217, 218, 374, and 478. The majority of 3A4 residues which interact with progesterone and/or erythromycin possess their equivalents in key residues of P450 2B enzymes, except for residues 297, 480 and 482, which do not contact either substrate in P450 3A4. The results from docking of progesterone and erythromycin into the enzyme model make it possible to pinpoint residues which may be important for 3A4 function and to target them for site-directed mutagenesis.

L16 ANSWER 31 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
STN DUPLICATE 8

ACCESSION NUMBER: 1996:758613 SCISEARCH Full-text  
THE GENUINE ARTICLE: VM600  
TITLE: Molecular modelling of CYP3A4 from an alignment with  
CYP102: Identification of key interactions between  
putative active site residues and CYP3A-specific chemicals  
AUTHOR: Lewis D F V (Reprint); Eddershaw P J; Goldfarb P S; Tarbit  
M H  
CORPORATE SOURCE: UNIV SURREY, SCH BIOL SCI, MOL TOXICOL GRP, GUILDFORD GU2  
5XH, SURREY, ENGLAND (Reprint); GLAXO WELLCOME RES & DEV  
LTD, BIOANAL & DRUG METAB DIV, WARE SG12 ODP, HERTS,  
ENGLAND  
COUNTRY OF AUTHOR: ENGLAND  
SOURCE: XENOBIOTICA, (OCT 1996) Vol. 26, No. 10, pp. 1067-1086.  
ISSN: 0049-8254.  
PUBLISHER: TAYLOR & FRANCIS LTD, ONE GUNPOWDER SQUARE, LONDON,  
ENGLAND EC4A 3DE.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 52  
ENTRY DATE: Entered STN: 1996  
Last Updated on STN: 1996

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB 1. A structural model of CYP3A4 is reported on the basis of a novel amino acid  
sequence alignment between the CYP3 family and CYP102, a bacterial P450 of known  
crystal structure. 2. Construction of the CYP3A4 model from CYP102 is facilitated by  
the relatively high sequence homology between the two proteins (52 % homology; 27 %  
identity) with many conservative amino acid changes, yielding a structure of low  
internal energy.  
3. A considerable number of specific substrates, and some specific inhibitors, are  
shown to occupy the putative CYP3A4 active site via interactions with the same amino  
acid residues in almost all cases investigated.  
4. The CYP3A4 model rationalizes the known positions of metabolism for many  
substrates of this major human P450 such that the route of metabolism in novel  
development compounds can be predicted.

L16 ANSWER 32 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1995:815639 SCISEARCH Full-text  
THE GENUINE ARTICLE: TG920  
TITLE: PROSTACYCLIN AND THROMBOXANE SYNTHASES  
AUTHOR: TANABE T (Reprint); ULLRICH V  
CORPORATE SOURCE: NATL CARDIOVASC CTR, RES INST, DEPT PHARMACOL, SUITA,  
OSAKA 565, JAPAN (Reprint); UNIV KONSTANZ, FAC BIOL,  
D-78434 CONSTANCE, GERMANY  
COUNTRY OF AUTHOR: JAPAN; GERMANY  
SOURCE: JOURNAL OF LIPID MEDIATORS AND CELL SIGNALLING, (OCT 1995)  
Vol. 12, No. 2-3, pp. 243-255.  
ISSN: 0929-7855.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE

LANGUAGE: English  
REFERENCE COUNT: 67  
ENTRY DATE: Entered STN: 1995  
Last Updated on STN: 1995  
=> log y